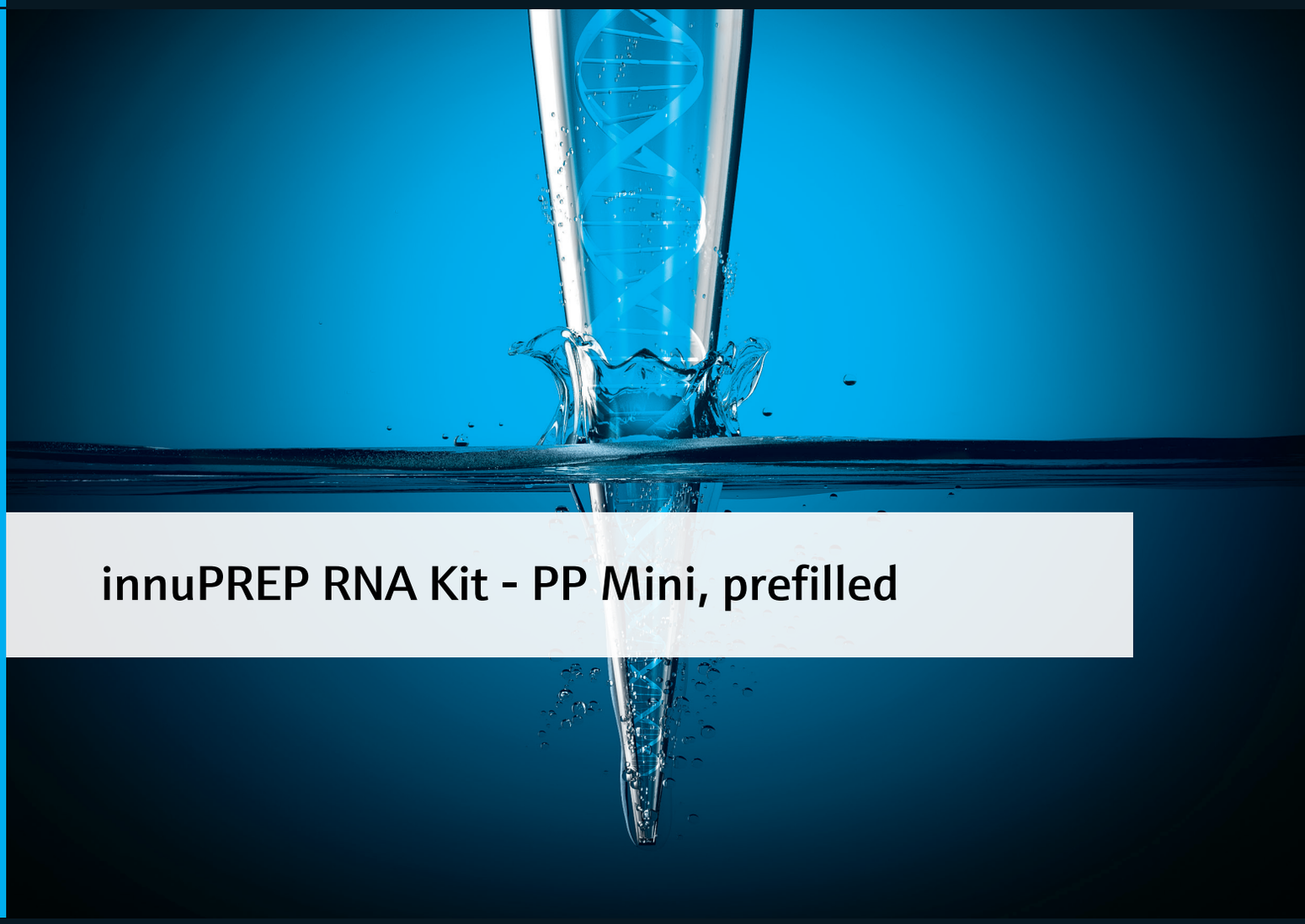
Instructions for Use
Life Science Kits & Assays



innuPREP RNA Kit - PP Mini, prefilled

Innuscreen GmbH

Order No.:

845-PPA-1660016	16 reactions
845-PPA-1660096	96 reactions
845-PPB-1660016	16 reactions
845-PPB-1660096	96 reactions

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This documentation describes the state at the time of publishing.
It needs not necessarily agree with future versions. Subject to change!

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1 Introduction

1.1 Intended use

The **innuPREP RNA Kit – PP Mini, prefilled** has been designed for automated isolation of total RNA from tissue samples, eukaryotic cells, bacterial cell pellets and yeasts.

The Reagent Plate / Strip of the kit is prefilled with almost all extraction reagents which are needed for the extraction process. After addition of the lysed sample (external lysis), DNase and MAG Suspension F, the automated extraction process begins by binding of RNA to surface-modified magnetic particles. After the washing steps, the RNA is eluted from the magnetic particles with RNase-free Water and is now ready to use for downstream applications.

The extraction procedure takes place on the magnetic particle processor PurePrep Mini and allows the parallel and flexible extraction of 1 up to 16 samples. Additionally, the kit is compatible with the PurePrep Midi device too. Please refer to the corresponding chapters of the devices.

The kit is intended for use by professional users. The RNA extracted using this kit is suitable for a wide range of downstream applications, such as amplification reactions and further analytical procedures.

CONSULT INSTRUCTION FOR USE

This package insert must be read carefully before use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.2 Notes on the use of this manual and the kit

For easy reference and orientation, the manual and labels use the following warning and information symbols as well as the shown methodology:

Symbol	Information
REF	**REF** Catalogue number.
Σ N	**Content** Contains sufficient reagents for <N> reactions.
15 °C 30 °C	**Storage conditions** Store at room temperature or shown conditions respectively.
	Consult instructions for use This information must be observed to avoid improper use of the kit and the kit components.
	Expiry date
LOT	**Lot number** The number of the kit charge.
	Manufactured by Contact information of manufacturer.
	For single use only Do not use components for a second time.
	Note / Attention Observe the notes marked in this way to ensure correct function of the kit and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → „Notes on the use of this manual“ p. 4).
- Working steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully before use to guarantee your own safety and a trouble-free operation.
Follow all the safety instructions explained in the manual, as well as all messages and information, which are shown.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

FOR SINGLE USE ONLY!
This kit is made for single use only!

ATTENTION!
Don’t eat or drink components of the kit!
The kit shall only be handled by educated personnel in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries. This kit could be used with potential infectious samples. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

Please observe the federal, state and local safety and environmental regulations. Follow the usual precautions for applications using extracted nucleic acids. All materials and reagents used for DNA or RNA isolation should be free of DNases or RNases.

ATTENTION!
Do not add bleach or acidic components to the waste after sample preparation!

NOTE

Emergency medical information in English and German can be obtained 24 hours a day from:

Poison Information Center, Freiburg / Germany

Phone: +49 (0)761 19 240.

For more information on GHS classification and the Safety Data Sheet (SDS) please contact sds.innu@ist-ag.com.

3 General notes and safety recommendations on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases have to be reduced to a minimum in accordance with the following recommendations:

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contaminations from surface of the skin or from dusty laboratory equipment.
- Change gloves frequently and keep tubes closed.
- Keep isolated RNA on ice.
- Reduce preparation time as much as possible.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase-free.)
- Non-disposable plastic ware should be treated before use to ensure that it is RNase-free. Plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. You can also take chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.

- All glassware should be treated before use to ensure that it is RNase-free. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240 °C for four hours or more before use. Autoclaving will not inactivate RNase activity completely. Oven baking inactivates RNases and ensures that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1 % DEPC (diethyl pyrocarbonate). The glassware has to be immersed in 0.1 % DEPC solution for 12 hours at 37 °C followed by autoclaving or heating to 100 °C for 15 minutes to remove residual DEPC.
- Electrophoresis tanks should be cleaned with detergent solution (e.g. 0.5 % SDS), thoroughly rinsed with RNase-free water, rinsed with ethanol and finally allowed to dry.
- All buffers have to be prepared with DEPC-treated RNase-free water.
- Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification will be performed.
- Do not use equipment, glassware and plastic ware employed for other applications which might introduce RNase contaminations in the RNA isolation.

4 Storage conditions

All kit components are shipped at ambient temperature.

Upon arrival store **DNase I and DNase I Digestion Buffer** at -22 to -18 °C and **MAG Suspension F** at 4 °C to 8 °C.

All other components of the kit should be stored dry at room temperature (15 °C to 30 °C). When stored at room temperature, the kit is stable until the expiration date printed on the label on the kit box.

If there are any precipitates within the provided solutions solve these precipitates by careful warming. Before every use make sure that all components have room temperature.

5 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This product has been produced and tested in an ISO 13485 certified facility.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the **innuPREP RNA Kit – PP Mini, prefilled** or other IST Innuscreen GmbH products, please do not hesitate to contact us. For technical support or further information in Germany please contact info.innu@ist-ag.com. For other countries please contact your local distributor.

6 Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Product specifications“ p. 10). Since the performance characteristics of IST Innuscreen GmbH kits have just been validated for the application described above, the user is responsible for the validation of the performance of IST Innuscreen GmbH kits using other protocols than those described below. IST Innuscreen GmbH kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA’ 88 regulations in the U.S. or equivalents in other countries.

All products sold by IST Innuscreen GmbH are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE

This kit is for research use only!

7 Kit components

7.1 Components included in the kit

	Σ 16	Σ 96
REF	845-PPA-1660016 [a] 845-PPB-1660016 [b]	845-PPA-1660096 [a] 845-PPB-1660096 [b]
Lysis Solution RP	10 ml	50 ml
MAG Suspension F	0.25 ml	1.1 ml
DNase I	75 µl	4 x 75 µl
DNase I Digestion Buffer	2 x 1.5 ml	10 x 1.5 ml
Reagent Plate B (PP) [a]	1	6
Reagent Strip B (PP) [b]	16	96
Tip Combs [a]	2	12
Tip Combs [b]	4	24
Manual	1	1

7.2 Components not included in the kit

- 1.5 ml tubes
- optional further Tip Combs (12 pcs) (31-01888, IST Innuscreen)

7.3 Components needed for isolation from bacteria (not included)

- Lysozyme (stock solution 10 mg/ml (400 U/µl))
- Mutanolysin (stock solution 0.4 U/µl)
- Lysostaphin (stock solution 0.4 U/µl)
- 1 x TE-Buffer

Alternatively:

- innuPREP Bacteria Lysis Booster (845-KA-1000050, 50 rxn, IST Innuscreen GmbH)

7.4 Components needed for isolation from yeasts (not included)

- Yeast Digest Buffer (50 mM KH_2PO_4, 10 mM DTT, pH 7.5)
- Lyticase (stock solution 10 U/µl)

8 Product specifications

1. Starting material:

- Tissue sample (up to 20 mg)
- Eukaryotic cells (max. 5 x 10^6)
- Bacteria / Yeast cell pellets (1 x 10^8 – 1 x 10^{12} cells)

2. Time for automated extraction protocol on PurePrep Mini / Midi:

- Approx. 40 minutes (excluding external lysis)

9 Initial steps before starting

- Prepare DNase I Digestion Solution according to the number of samples

Component	8 samples	16 samples	n samples
DNase I	30 µl	54 µl	3 µl x n+2
DNase I Digestion Buffer	1.37 ml	2.5 ml	137 µl x n+2
Final volume	1.4 ml	2.55ml	140 µl x n+2

- Avoid freezing and thawing of starting material.

10 Preparing Reagent Plate / Strip for automated extraction

10.1 General filling scheme of the reagents (already prefilled)

Cavity of a DW Plate/Strip	Content
Cavity 1 + 7	empty
Cavity 2 + 8	400 µl Isopropanol
Cavity 3 + 9	800 µl Washing Solution HS
Cavity 4 + 10	800 µl Washing Solution LS
Cavity 5 + 11	800 µl Washing Solution LS
Cavity 6 + 12	100 µl RNase-free Water

10.2 Unpacking of Reagent Plate and peeling off the sealing foil

Reagent Plates and Reagent Strips are delivered wrapped into plastic bags for transport protection.

Carefully open the overpack of Reagent Plates/Strips by using scissors.

Afterwards gently remove the foil by peeling off.

11 Sample Preparation

11.1 Protocol 1: RNA extraction from tissue samples

NOTE

To maximize the final yield of total RNA a complete homogenization of tissue sample is important! For the homogenization of tissue samples, it is possible to use a commercially available homogenizer for bead beating. It is also possible to disrupt the starting material using mortar and pestle in liquid nitrogen and grind the tissue sample to a fine powder.

For longer storage place the sample in **Lysis Solution RP** at -22 °C to -18 °C.

A. Homogenization of tissue samples using a homogenizer for bead beating

1. Transfer the weighed amount of fresh or frozen starting material into a suitable reaction vessel for the homogenizer. (We recommend using IST Innuscreen's innuSPEED Lysis Tubes)
2. Add **450 µl Lysis Solution RP**.
3. Homogenize the sample.
4. Transfer the homogenized tissue sample into a 1.5 ml reaction tube and incubate the sample for 10-30 minutes (depending on sample size) at room temperature under continuous shaking (~550 rpm).
5. After lysis spin down unlysed material by centrifugation at maximum speed for 1 minute.
6. Proceed with "Automated extraction using PurePrep Mini" on p.15 or "Automated extraction using PurePrep Midi" p.17.

B. Disruption of the tissue sample using a mortar and pestle and liquid nitrogen

1. Transfer the weighed amount of fresh or frozen starting material under liquid nitrogen and grind the material to a fine tissue powder.
2. Transfer the powder into a 1.5 ml reaction tube. Don't allow the sample to thaw!
3. Add **450 µl Lysis Solution RP** and incubate the sample for 10 - 30 minutes (depending on sample size) at room temperature under continuous shaking (~550 rpm).
4. After lysis spin down unlysed material by centrifugation at maximum speed for 1 minute.
5. Proceed with "Automated extraction using PurePrep Mini" on p.15 or "Automated extraction using PurePrep Midi" p.17.

11.2 Protocol 2: RNA extraction from eukaryotic cells

1. Add **400 µl Lysis Solution RP** to the cell pellet and incubate for 2 minutes at room temperature. Resuspend the cell pellet completely by pipetting up and down.
2. Incubate the sample at room temperature under continuous shaking (~550 rpm) for 10 minutes.
3. After lysis spin down unlysed material by centrifugation at maximum speed for 1 minute.

NOTE

To maximize the final yield of total RNA a complete disruption and lysis of the cell pellet is important! No cell clumps should be visible after lysis step.

4. Proceed with "Automated extraction using PurePrep Mini" on p.15 or "Automated extraction using PurePrep Midi" p.17.

11.3 Protocol 3: RNA extraction from bacteria and yeasts

1. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 10 minutes at 3,000 x g). Discard the supernatant.

   Do not discard the pellet!

2. Resuspend the bacterial cell pellet in **200 µl TE Buffer** and the yeast cell pellet **in 200 µl Yeast Digest Buffer** (→ see "Components needed for isolation from yeasts", p. 10) in a 1.5 ml tube and add the enzymes according to the table below or follow the instruction of the product **innuPREP Bacteria Lysis Booster**:

	Enzyme	Volume
Gram-negative bacteria	Lysozyme: 10 mg/ml (400 U/µl)	20 µl
Staphylococcus spp.	Lysostaphin: 0.4 U/µl	10 µl
Gram-positive bacteria	Lysozyme: 10 mg/ml (400 U/µl) + Mutanolysin: 0.4 U/µl	20 µl + 5µl
Yeast	Lyticase: 10 U/µl	10 µl

3. Incubate at 37 °C for 30 min under continuous shaking (~550 rpm).
4. Add **200 µl Lysis Solution RP** to the pre-lysed sample and vortex vigorously or pipette sometimes up and down.
5. Incubate the sample at RT under continuous shaking for 10 minutes.
6. After lysis spin down unlysed material by centrifugation at maximum speed for 1 minute.

NOTE

To maximize the final yield of total RNA a complete disruption and lysis of the cell pellet is important! No cell clumps should be visible after lysis step.

7. Proceed with "Automated extraction using PurePrep Mini" on p.15 or "Automated extraction using PurePrep Midi" p.17.

12 Automated extraction using PurePrep Mini

12.1 Prefilled DW Plate or the DW Strips

	1	2	3	4	5	6	7	8	9	10	11	12
A	DNase Digest	Sample 1	→	→	→	Eluate 1	DNase Digest	Sample 9	→	→	→	Eluate 9
B		Sample 2	→	→	→	Eluate 2		Sample 10	→	→	→	Eluate 10
C		Sample 3	→	→	→	Eluate 3		Sample 11	→	→	→	Eluate 11
D		Sample 4	→	→	→	Eluate 4		Sample 12	→	→	→	Eluate 12
E		Sample 5	→	→	→	Eluate 5		Sample 13	→	→	→	Eluate 13
F		Sample 6	→	→	→	Eluate 6		Sample 14	→	→	→	Eluate 14
G		Sample 7	→	→	→	Eluate 7		Sample 15	→	→	→	Eluate 15
H		Sample 8	→	→	→	Eluate 8		Sample 16	→	→	→	Eluate 16

Fig. 1: Schematic illustration of DW Plate

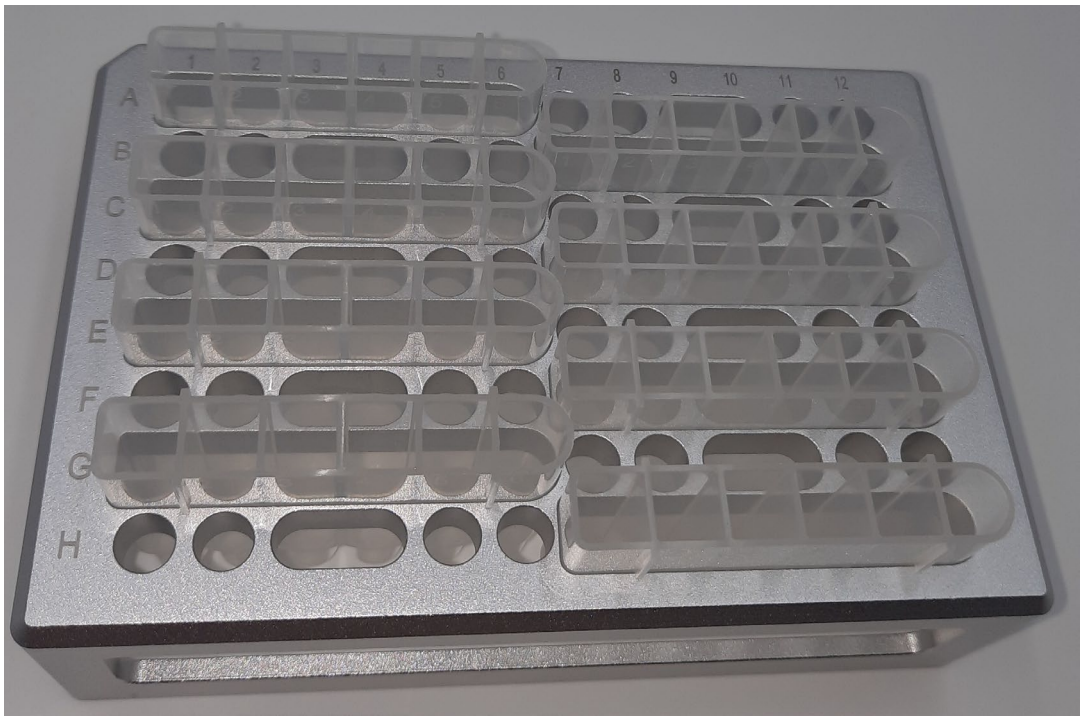

Fig. 2: Arrangement of the DW Strips in Tray

12.2 Loading filled Deep Well Plate/Strips to the PurePrep Mini and plug in the Tip Combs

1. Add 140 µl DNase I Digest Solution (see step 9) to cavity 1 and/or 7 of the Deep Well Plate/Strip.
2. Add **400 µl of the lysed sample** and **10 µl** of well mixed **MAG Suspension F** to cavity 2 and/or 8 of the Deep Well Plate/Strip.

NOTE

Mix the **MAG Solution F** well by vortexing for 1 minute.

3. Load the prefilled Plate / Strip to the PurePrep Mini and afterwards the TipCombs.

NOTE

- When using strip (strips), the strip is inserted into the tray. In total, a maximum of 8 strips can be used in one extraction-run.
- When working with strips, only every second tip is being used for extraction:

  Left tray side: Tip 1, 3, 5, 7

  Right tray side: Tip 2, 4, 6, 8.
- It is recommended to mark the tips used for the extraction so that they are not used more than once.

4. Select the protocol “RNAheat” and start the run.
5. After finishing the extraction protocol, the cavities 6 and 12 contain the isolated RNA.
6. Transfer the RNA into a fresh 1.5 ml Tube.

IMPORTANT NOTE

Store the RNA under adequate conditions.

We recommend storing the extracted RNA at −82 °C to −78 °C.

13 Automated extraction using PurePrep Midi

NOTE

According to the chapter of the PurePrep Mini the illustration of the plates will be the same!

The prefilling is carried out from left to right as shown in the illustration, Fig. 1. For the PurePrep Midi, 2 DW Plates can be loaded in parallel to run 32 samples.

13.1 Loading filled Deep Well Plate to the PurePrep Midi and plug in the Tip Combs

NOTE

It is recommended to mark the tips used for the extraction so that they are not used more than once.

1. Add 140 µl DNase I Digest Solution (see step 9) to cavity 1 and/or 7 of the Deep Well Plate/Strip.
2. Add 4**00 µl of the lysed sample** and **10 µl** of well mixed **MAG Suspension F** to cavity 2 and/or 8 of the Deep Well Plate/Strip.

NOTE

Mix the **MAG Solution F** well by vortexing for 1 minute.

3. Load the prefilled Plate / Strip to the PurePrep Midi and the TipCombs afterwards.
4. Select the protocol “RNAheat“ and start the run.
5. After finishing the extraction protocol, the cavities 6 / 12 contains the isolated RNA.
6. Transfer the RNA into a fresh 1.5 ml Tube.

IMPORTANT NOTE

Store the RNA under adequate conditions.

We recommend storing the extracted RNA at −82 °C to −78 °C.

14 Troubleshooting

Problem / probable cause	Comments and suggestions
Poor lysis of starting material	
Insufficient disruption or homogenization.	After lysis centrifuge lysate to pellet debris and continue with the protocol using the supernatant. Reduce amount of starting material.
Insufficient lysis time.	Increase length of lysis step.
Little or no total RNA eluted	
Insufficient disruption or homogenization	Reduce amount of starting material. Overloading reduces yield!
DNA contamination	
Too much starting material	Reduce amount of starting material or increase volume of DNase I.
Incorrect lysis of starting material	Use the recommended techniques for lysis of cell pellet.
Total RNA degraded	
RNA source inappropriately handled or stored	Ensure that the starting material is fresh! Ensure that the protocol, especially the first steps, has been performed quickly.
RNase contamination of solutions, etc.	Use sterile, RNase-free filter tips. Before every preparation clean up the pipette, the devices and the working place. Always wear gloves!
Lysis step too long.	Reduce length of lysis step.

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